SPECIFICATION AMENDMENTS

EXAMPLE 1

In Vitro Assay

In vitro binding assay for non-peptide CCR1 receptor antagonists

This assay demonstrates the affinities of the non-peptide CCR1 receptor antagonists of the invention, preferably a non-peptide CCR1 receptor antagonist of formula (I), for binding to the rat CCR1 receptor.

Reagents and Solutions:

Chemokines: MIP-1α and RANTES (Peprotech Inc.)

Cells: Rat peripheral blood mononuclear cells (PBMC) were isolated from whole blood from Lewis rats by ~~Accu-Paque™~~ ACCU-PAQUE™ (4.2% w/v dextran and 11.6% w/v sodium diatrizoate) (Accurate Chemical & Scientific Corp.) density centrifugation.

Ligand: ^{125}I-MIP-1α and ^{125}I-RANTES from New England Nuclear (specific activity is 2200 Ci/mmol, 25 µCi/vial) was reconstituted in 1 mL H_2O.

Assay buffer: 130 mM NaCl, 5 mM KCl, 1 mM $MnCl_2$, 50mM Tris, 30 µg/ml bacitracin, 0.1% BSA, pH 7.4.

Wash buffer: Phosphate buffer solution (PBS)

Compounds of the Invention: The stock solution of the compounds was 1 mM in 100% DMSO. The highest concentration in the assay was 10 µM and may vary depending on the potency of the compounds. Serial 1:3 dilutions from the highest concentration were made with assay buffer. Six concentrations of each compound were typically screened to generate a dose curve from which the K_i value was determined.

Assay procedure:

Assays were performed in 96-well v-bottom microtiter plates in a total volume of 100 µL.

Rat PBMC were washed once in PBS and resuspended in the assay buffer to about 0.2 to 1.0 x 10^6 cells/mL. Cells were incubated with ^{125}I-MIP-1α or ^{125}I-RANTES in the presence or absence of varying concentrations of unlabeled MIP-1α, RANTES, or compound at 4°C for 30 minutes.

The reactions were terminated by removing aliquots from the cell suspension and separating cells from buffer by centrifugation through a silicon/paraffin oil mixture as described in Hesselgesser *et al.*, (1998), *supra*.

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The nonspecific binding was determined in the presence of 100 nM or 1 μM of unlabeled MIP-1α or RANTES. The concentrations of compounds in the assay were typically from 10 μM to 30 nM in 1:3 dilutions and the concentrations for more potent compounds were lower depending on the potency.

Calculations:

The dose curves of each compound with 6 concentration points were generated and the binding data were curve fitted with the computer program IGOR (Wavemetrics) to determine the affinity and number of sites.

The non-peptide CCR1 receptor antagonists of the invention, when tested in this assay, demonstrated their affinity to bind to the rat CCR1 receptor.

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